

REPRODUCTIVE BIOLOGY OF PIRIQUETA CAROLINIANA (TURNERACEAE)

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When Charles Darwin published his classic "Forms of Flowers" (1877) he summarized the information then available concerning heterostyly in various plant families. At that time the Turneraceae was missing from his list, although a few years later Urban (1883) reported that about 70% of the members of this family were distylous. Despite this early documentation of the prevalence of heterostyly in the Turneraceae, few investigations have been made on the reproductive method of any of its species.

The Turneraceae is the only family in the large Englerian order Parietales in which heterostyly seems definitely to be present, although there is an unconfirmed report for the Guttiferae (Thiselton-Dyer, 1872). Because of the isolated occurrence of floral heteromorphism in this order, an investigation of the breeding system of heterostylous species of Turneraceae is warranted. Furthermore, certain authors have described each of the floral forms of heterostylous species in other families as separate species (discussed by Hildebrand, 1866; Ray and Chisaki, 1957). Other authors have misinterpreted floral dimorphism as dioecism (Darwin, 1877). Our purpose, therefore, is to record our observations on the reproductive biology of *Piriqueta caroliniana*, to establish that both flower forms belong to the same species, and to demonstrate that both are necessary for sexual reproduction to occur.

Piriqueta caroliniana (Walt.) Urban is a polytypic species which occurs in South America, the West Indies, and the southeastern United States. At least 4 of its described varieties occur in Florida, but the only variety we have investigated is *P. caroliniana* var. *caroliniana*, which occurs as far north as South Carolina. There is a report of the species in North Carolina (Urban, 1883, and subsequent authors), but we have seen no specimens collected or definitely cited from that state.

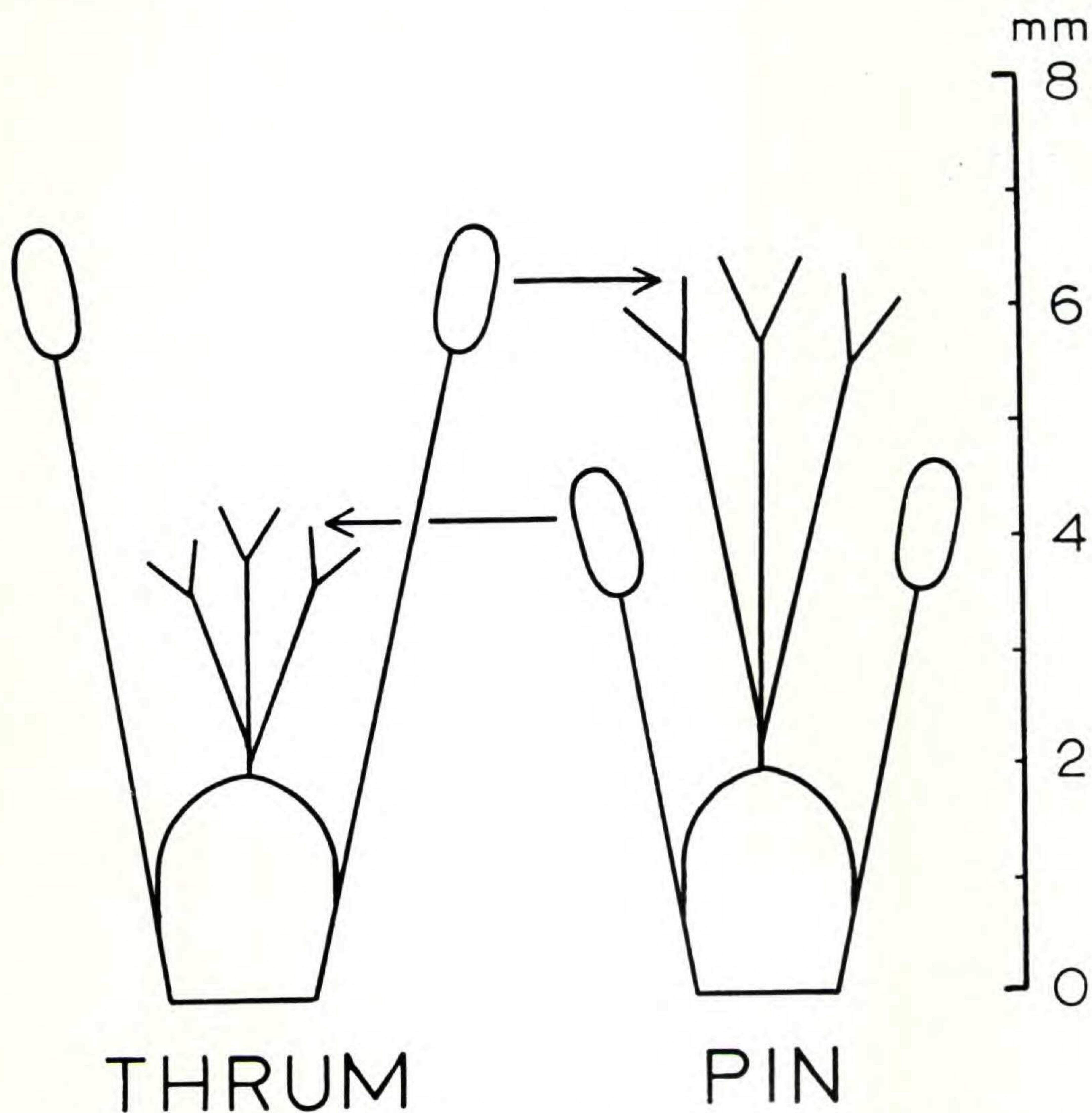


Fig. 1. Diagrammatic representation of the 2 flower forms and compatibility relationships of *Piriqueta caroliniana*. Arrows from anthers to stigmas indicate the direction of the most compatible crosses. All other pollinations are comparatively incompatible.

The species occurs in sandy soils of dunes, open pine or hardwood forests, and grassy areas of the Coastal Plain. Flowering begins in late spring and continues through the summer, although specimens from Florida apparently produce occasional flowers at almost any time of the year. The golden-yellow corollas open in early morning and remain open until mid-afternoon, at which time they close and wither. The plants are long-lived perennials which reproduce not only by seed, but by means of numerous shoots which develop vegetatively from the roots, often at a con-

siderable distance from the parent plant. The arillate seeds are probably dispersed by ants (Brizicky, 1961). In areas where it is abundant, *P. caroliniana* forms large and showy colonies.

Two kinds of plants occur in populations of *P. caroliniana*. One of these bears flowers with long styles and short stamens ("pin" flowers) and the other bears flowers with short styles and long stamens ("thrum" flowers, Fig. 1). Early observers considered floral dimorphism (in other families) to be a structural adaptation which promotes cross pollination. Insects visiting these flowers would carry pollen from the high anthers of one flower to the high stigma of another, and from the low anthers to low stigmas. When several of these species were investigated it was demonstrated that incompatibility systems promoting outbreeding were usually associated with this floral heteromorphism (Darwin, 1877). Thus, what was originally a morphological term (heterostyly) acquired physiological connotations as well. There are, however, numerous examples known in which heterostyly is not associated with an incompatibility system, so that outbreeding in these species must depend primarily on the positional relationships of the anthers and stigmas of the different flower forms. We have attempted to determine both the morphological and physiological features of distyly in *P. caroliniana*.

Measurements of floral parts of preserved flowers and of fresh pollen grains mounted in lacto-phenol are given in Table 1. These figures indicate complete dimorphism of *P. caroliniana* with respect to style length, stamen length, and size of pollen grains. Particularly noteworthy is the good reciprocal correspondence in the heights of anthers and stigmas of the 2 forms (Fig. 1). Occasional overlap in individual measurements for the height of these organs in the 2 forms occurred, but the averages and modes are distinct. Average pollen-grain diameter of the thrum flowers is 13.8μ greater than that of the pin flowers. Although substantial overlap in individual measurements of pollen grains occurred, the average figures for the 15 pin plants and the 10 thrum plants sampled were mutually exclusive for the 2 forms.

Table 1. Measurements of floral parts and pollen grains of *Piriqueta caroliniana* var. *caroliniana*

Form	Height (mm) of stigma above ovary base	Height (mm) of anther above ovary base	Pollen grain diameter (μ)
Long	6.4 (5.0-8.0)	4.5 (4.0-5.5)	59.4 (52.0-72.8)
Short	4.2 (3.5-5.0)	6.9 (5.0-8.0)	73.2 (62.4-83.2)

In a distylous species, the possible pollinations which may occur are (1) self-pollinations or own-form pollinations between different plants of the same floral form and (2) cross pollinations between the 2 floral forms. Pollinations in the first category are termed “illegitimate” and the second are “legitimate” (Darwin, 1877). Plants of *P. caroliniana* were transplanted to the Duke University greenhouses from populations in Allendale and Orangeburg counties, South Carolina. An extensive crossing program was carried out with these plants to determine the pollen-carpel compatibility relationships in the 2 forms. Numbered plants were placed in insect proof cages and artificially pollinated in various combinations. Records of capsule and seed production of individual plants were kept, but the results of each type of cross have been combined and presented in summary form (Table 2). While the results of these pollinations are valid for comparative purposes, it should be mentioned that the low percentage of capsules produced by the most fertile crosses must be attributed to pollinations carried out near the end of the flowering season of the plants. When it was realized that none of these late pollinations resulted in fruit production, the program was stopped.

The data presented in Table 2 suggest that the pin plants are strongly self-incompatible, although crosses between pin plants resulted in the production of a small number of well-filled capsules. Additional self-pollinations using other plants may reveal a greater similarity between the results of self-pollinations and own-form pollinations in the pin plants. The results of illegitimate pollinations of the thrum plants indicate a moderately strong self-incompatibility of this form and suggest that thrums may be somewhat more self-compatible than are the pins.

Table 2. Results of legitimate and illegitimate pollinations of *Piriqueta caroliniana* var. *caroliniana*.

Type of cross (♀ × ♂)	No. of plants used as ♀	Number of flowers pollinated	capsules produced	capsules collected	Number of seeds collected	Per cent of flowers producing capsules	Average number of seeds per capsule collected	Average number of seeds per pollination
Long × Long	12	284	45	39	496	15.8	12.7	1.7
Long selfed	3	21	0	0	0	0	0	0
Long × Short	15	218	114	95	1874	52.3	19.7	8.6
Short × Short	12	215	57	47	584	26.5	12.4	2.7
Short selfed	6	52	14	9	117	26.9	13.0	2.3
Short × Long	10	141	86	69	1382	61.0	20.0	9.8

Rhodora

The legitimate reciprocal pollinations between pin and thrum plants provide a strong contrast to the illegitimate pollinations. These legitimate cross pollinations were highly productive of capsules and seeds. Furthermore, pin and thrum plants were similar in the proportion of flowers which produced capsules, in the average number of seeds per total pollinations, and in the average number of seeds per capsule. The results of the legitimate and the illegitimate crosses demonstrate beyond doubt that an incompatibility system is linked with distyly in *Piriqueta caroliniana*.

The results presented above attest to the efficiency of a physiological incompatibility system which prevents certain fertilizations from occurring, but they do not account for the means of pollination under natural conditions. In the field *P. caroliniana* is freely visited by bees which collect nectar and pollen from the flowers. The insects visiting a population in Orangeburg County, S. C., were collected by the first author in late May, 1962, and identified by Dr. T. B. Mitchell, emeritus professor of entomology at North Carolina State College.

The collection consisted almost exclusively of bees; no Lepidoptera or Coleoptera were seen visiting the plants and only a few Diptera were noted. The most common bee visitor to the plants was *Halictus ligatus* Say (Halictidae), a widespread bee known to pollinate a number of genera (Mitchell, 1960). Also observed, but less abundant than the preceding species, were *H. parallelus* Say, *Colletes brevicornis* Robt. (Colletidae), and the newly-described *Dialictus brassicae* Mitch. (Halictidae). It is probable that additional bee species and members of other insect groups accomplish pollination as well.

In order to make a final determination of the combined efficacy of the incompatibility system, the floral dimorphism, and the pollinators of *P. caroliniana*, the following simple experiments were carried out. A flat with 6 pots of pin plants was set outside the university greenhouses and, at the same time, another flat with 6 pots of thrum plants was put outdoors at a site separated from the greenhouses by

about 1300 meters of dense forest. The nearest known natural colony of *P. caroliniana* occurs 300 miles to the south. The plants in these flats were visited by numerous insects, particularly species of *Halictus*. At the end of 2 weeks the plants were examined for capsules. Although well over 100 flowers had been produced by each group, not one capsule had developed. After these observations were made, the pots of pin and thrum plants were placed together outside the greenhouses. At the end of 2 weeks every flower which had developed during this period produced a full capsule. The average seed number per capsule produced by this open pollination method was 21.1, which compares favorably with the figures we obtained from the artificial legitimate pollinations (Table 2). These results not only attest to the efficiency of the insect pollinators, but to the effectiveness of the floral structure and incompatibility system in preventing seed production from illegitimate pollinations.

In most distylous species which have been studied the style and stamen characters of the 2 forms are controlled by a pair of allelic supergenes (Lewis, 1954). The pin is *ss* and the thrum is *Ss*. Some *SS* thrum plants may also occur in some self-compatible heterostylous species as a result of self-pollinations (Ray and Chisaki, 1957). In certain members of the Plumbaginaceae, pins are the heterozygotes and thrums are the homozygotes (Baker, 1954). With either genetic system, enforced outbreeding between pins and thrums would result in a 1:1 ratio of the 2 forms in the progeny of either form. Therefore, in natural populations of distylous species where crossing is exclusively between the 2 forms, as it is in *P. caroliniana*, the population composition should be in the ratio 1 pin : 1 thrum.

Although the genetic basis of heterostyly in *Piriqueta* is still unknown, the following information is pertinent to its eventual determination. Seed obtained from a single thrum plant in the Orangeburg population was planted and the style length of its progeny recorded as they flowered. This progeny consisted of 34 pins and 35 thrums, or a 1:1 ratio. A population of the species in Coffee County, Georgia, sampled by

Hardin (1953) consisted of 22 pins and 18 thrums. To these figures may be added 33 pins and 29 thrums for a population growing in Allendale County, S. C. Both samples probably represent a 1:1 ratio, although the slight excess of one form (if proved valid in larger samples) may be significant in light of Crosby's suggestion (1949) that deviations from 1:1 may result from excessive illegitimate pollinations of one form. The compatibility data for *P. caroliniana* suggest, however, that illegitimate pollinations of pins in the field should be less productive than those involving thrum plants. The information presented above does not establish the genetic basis for distyly in *Piriqueta*, but it does not contradict the possibility that a single pair of supergenes may control its expression, as is true of all other genera which have been studied.

In most features of its breeding system and floral morphology *Piriqueta caroliniana* closely parallels the patterns which have independently evolved in distylous species belonging to other distantly related families, such as the Primulaceae, Polygonaceae, Menyanthaceae, and Rubiaceae. The greater productivity of seeds and capsules from illegitimate pollinations of the thrum flowers than from illegitimate pollinations of the pin flowers is unusual, for in most other families this relationship is reversed (Darwin, 1877).

Little information has been published concerning the breeding systems in the Turneraceae. Lock (*vide* Brizicky, 1961) has reported that the heterostylous *Turnera subulata* J. E. Sm. (as *T. ulmifolia* var. *elegans*) is self-incompatible. Urban observed that homostylous plants of *T. ulmifolia* L. var. *angustifolia* Willd. (var. *ulmifolia* *vide* Brizicky, 1961) were self-compatible but frequently outcrossed by insects; our own observations on cultivated specimens of this variety confirm these observations. Urban (1883) has pointed out that in various genera of the Turneraceae species pairs exist in which one of the members is heterostylous, large-flowered, and perennial and the other is homostylous, small-flowered, and annual. One of these pairs consists of *Piriqueta caroliniana* and its close relative *P. cistoides* (L.) Griseb., an annual

homostylous species of South America and the West Indies. The breeding system of *P. cistoides* is not known, although by analogy with the behavior of the varieties of *Turnera ulmifolia* described above, and by examples in other genera with closely-related heterostylous and homostylous species (Ernst, 1955; Baker, 1959; Darwin, 1877) it is probably self-compatible. The descriptions of the flowers of various Turneraceae given by Urban (1883) suggest the occurrence of "incomplete" distyly as well as other variations upon the distylous pattern. The family is clearly one which merits further investigation by botanists interested in the comparative evolution of heteromorphic incompatibility systems.

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SUMMARY

Piriqueta caroliniana (Walt.) Urb. var. *caroliniana* is distylous. Its two forms differ from each other in style length, stamen length, and pollen size. Crossing experiments show that the pin (long-styled) and thrum (short-styled) plants are moderately to strongly self-incompatible, but the two forms are highly cross-compatible. In the field, plants are visited by various bees which are effective pollinators. Populations are composed of approximately 1 pin: 1 thrum, suggesting that the genetic basis of distyly in this species may be similar to models proposed for distylous genera of other families. Breeding systems of other Turneraceae are briefly discussed, and a report of self-compatibility in the homostylous *Turnera ulmifolia* L. var. *ulmifolia* is confirmed. *Piriqueta cistoides* (L.) Griseb. is closely related to *P. caroliniana*, but differs in its annual duration and its smaller homostylous flowers; it is probably self-compatible.

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